

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

760-281P *Pct*
U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/600602

INTERNATIONAL APPLICATION NO. PCT/JP99/05221	INTERNATIONAL FILING DATE September 24, 1999	PRIORITY DATE CLAIMED November 19, 1998
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TITLE OF INVENTION

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APPLICANT(S) FOR DO/EO/US

UEKI, Jun

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(3)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(2)).
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98/International Search Report with cited references
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
 - 1.) Sequence Listing (4 pages)
 - 2.) Zero (0) sheets of Formal Drawings

027 600602

PCT/JP99/05221

760-281P

- 17.
- ☒
- The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482)
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO
and International Search Report not prepared by the EPO or JPO. \$970.00

International preliminary examination fee (37 CFR 1.482) not paid to
USPTO but International Search Report prepared by the EPO or JPO. \$840.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO
but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. \$690.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO
but all claims did not satisfy provisions of PCT Article 33(1)-(4). \$670.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO
and all claims satisfied provisions of PCT Article 33(1)-(4). \$96.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

Surcharge of \$130.00 for furnishing the oath or declaration later than ☒ 20 ☐ 30
months from the earliest claimed priority date (37 CFR 1.492(e)).

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total Claims	24 - 20 =	4	X \$18.00
Independent Claims	3 - 3 =	0	X \$78.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable) Yes			+ \$260.00

TOTAL OF ABOVE CALCULATIONS =

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity statement
must also be filed (Note 37 CFR 1.9, 1.27, 1.28).

SUBTOTAL =

Processing fee of \$130.00 for furnishing the English translation later than ☐ 20 ☐ 30
months from the earliest claimed priority date (37 CFR 1.492(f)).

TOTAL NATIONAL FEE =

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be
accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +

TOTAL FEES ENCLOSED =

Amount to be:

refunded \$

charged \$

- a. ☒ A check in the amount of \$ **1432.00** to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account. No. _____ in the amount of \$ _____ to cover the above fees.
A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
overpayment to Deposit Account No. 02-2448.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:

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SIGNATURE

for MURPHY, GERALD M., JR.
NAME

#28,977 (GMM)

REGISTRATION NUMBER

09/600602

532 Rec'd PCT/PTC 19 JUL 2000

PATENT
760-281P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: UEKI, Jun
Int'l. Appl. No.: PCT/JP99/05221
Appl. No.: New Group:
Filed: July 19, 2000 Examiner:
For: NUCLEIC ACID FRAGMENTISM RECOMBINANT VECTORS
CONTAINING THE SAME AND METHOD FOR PROMOTING
EXPRESSION OF STRUCTURAL GENES USING THE SAME

PRELIMINARY AMENDMENT**BOX PATENT APPLICATION**

Assistant Commissioner for Patents
Washington, DC 20231

July 19, 2000

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTSIN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/JP99/05221 which has an International filing date of September 24, 1999, which designated the United States of America.--

IN THE CLAIMS:

Please amend the claims as follows:

Claim 5: Line 2, change "any one of claims 1-4" to
--claim 1--

Claim 10: Line 1, change "any one of claims 6 to 9" to
--claim 6--

Claim 17: Line 1, change "any one of claims 13 to 16" to
--claim 13--

Claim 19: Line 1, change "any one of claims 13 to 18" to
--claim 13--

Claim 20: Line 2, change "any one of claims 13 to 19" to
--claim 13--

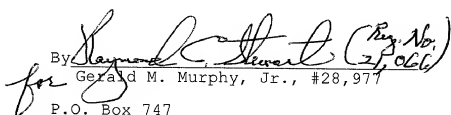
REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application. The claims have also been amended to delete the improper multiple dependencies and to place the application into better form for examination. Entry of the present amendment and favorable action on the above-identified application are respectfully requested.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By  (Reg. No. 27,066)
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GMM/cgc
760-281P

SPECIFICATION

Nucleic Acid Fragments, Recombinant Vectors Containing the Same and Method for Promoting Expression of Structural Genes Using the Same

Technical Field

5 The present invention relates to a nucleic acid fragment having the function to promote expression of structural genes located downstream of the nucleic acid fragment, a recombinant vector containing the same and to a method for expression of structural genes using the same.

Background Art

10 Promotion of expression of foreign genes is the most required technique for applying the genetic engineering techniques to plants. This technique includes utilization of a DNA fragment having an activity to promote gene expression. Known DNA fragments which promote expression of foreign genes include the intron of maize alcohol dehydrogenase (Callis et al. Gene & Development 1, 1183-
15 1200 (1987)), and the first intron of rice phospholipase D (hereinafter also referred to as "PLD") (International Publication WO96/30510). Further, the influences on the activity to promote gene expression, by deleting a part of an internal region of an intron or by inserting the same intron into a site within the intron, have been reported (Mascarenhas et al. Plant Mol. Biol. 15, 913-920 (1990), Clancy et al. Plant Sci. 98,
20 151-161 (1994)).

 However, so far, the number of DNA fragments which may be used for this purpose is limited, and in most cases, their gene expression-promoting effects are insufficient. Therefore, a DNA fragment having higher activity has been demanded. Further, although it has been tried to increase the expression-promoting activity by
25 modifying the intron sequences, the region having the activity to promote expression in an intron has not been reported, and a case wherein the promotion activity of an original intron-originated DNA fragment is doubled is not known.

Disclosure of the Invention

Accordingly, an object of the present invention is to provide a novel nucleic acid fragment having a high activity to promote expression of structural genes located downstream of the nucleic acid fragment; a recombinant vector containing the above-mentioned nucleic acid fragment, in which expression of a structural gene is promoted; and to provide a method for promoting expression of a structural gene using the above-mentioned nucleic acid fragment, which structural gene is located downstream of the nucleic acid fragment.

The present inventor intensively studied to discover that a specific region in the first intron of rice phospholipase D (hereinafter also referred to as "PLD") has a high activity to promote gene expression, thereby completing the present invention.

That is, the present invention provides an isolated nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO: 1 in Sequence Listing or an isolated nucleic acid fragment (excluding the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 3 in Sequence Listing) having the same nucleotide sequence as shown in SEQ ID NO: 1 except that one or a plurality of nucleotides are substituted or deleted, or except that one or a plurality of nucleotides are inserted or added, which has an activity to promote expression of a structural gene located downstream of said nucleic acid fragment. The present invention also provides a recombinant vector comprising at least a nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO: 1 in Sequence Listing or a nucleic acid fragment (excluding the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 3 in Sequence Listing) having the same nucleotide sequence as shown in SEQ ID NO: 1 except that one or a plurality of nucleotides are substituted or deleted, or except that one or a plurality of nucleotides are inserted or added, which has an activity to promote expression of a structural gene located downstream of said nucleic acid fragment, and a structural gene located downstream of said nucleic acid fragment,

whose expression is promoted by said nucleic acid fragment. The present invention further provides a method for promoting expression of a structural gene, comprising inserting, at a location upstream of said structural gene, a nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO: 1 in Sequence Listing or a nucleic acid fragment (excluding the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 3 in Sequence Listing) having the same nucleotide sequence as shown in SEQ ID NO: 1 except that one or a plurality of nucleotides are substituted or deleted, or except that one or a plurality of nucleotides are inserted or added, which has an activity to promote expression of a structural gene located downstream of said nucleic acid fragment. The present invention further provides a plant in which expression of a desired structural gene is promoted by the method according to the present invention as well as progenies thereof retaining the character.

By the present invention, a novel nucleic acid fragment which significantly promotes expression of a structural gene by inserting the nucleic acid into a site upstream of the structural gene was provided. By inserting the nucleic acid fragment according to the present invention into a site upstream of a structural gene, expression of the structural gene is promoted. Therefore, by the present invention, expression of, for example, a foreign gene in a recombinant vector may be promoted, so that it is expected that the present invention will largely contribute to the field of genetic engineering or the like.

Best Mode for Carrying Out the Invention

As mentioned above, the nucleic acid fragment according to the present invention is the nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO: 1 in Sequence Listing or the nucleic acid fragment having the same nucleotide sequence as shown in SEQ ID NO: 1 except that one or a plurality of nucleotides are substituted or deleted, or except that one or a plurality of nucleotides are inserted or added, which has an activity to promote expression of a structural

gene located downstream of the nucleic acid fragment. However, the nucleotide sequence shown in SEQ ID NO: 3 in Sequence Listing is the nucleotide sequence of the first intron of rice PLD, and since it has been disclosed by the present inventor that the first intron of rice PLD has an activity to promote expression of the gene located downstream thereof (International Publication WO96/30510), this sequence is excluded. The nucleotide sequence shown in SEQ ID NO:1 is the nucleotide sequence of the region in the first intron (SEQ ID NO: 3) of rice PLD from the second nucleotide (hereinafter indicated such as "2 nt") from the 5'-end to 65 nt.

As mentioned above, the nucleic acid fragments (hereinafter also referred to as "modified nucleic acid fragment" for convenience) having the same nucleotide sequence as shown in SEQ ID NO: 1 except that one or a plurality of nucleotides are substituted or deleted, or except that one or a plurality of nucleotides are inserted or added, which have activities to promote expression of a structural gene located downstream of said nucleic acid fragments are also within the scope of the present invention. In this case, the region in the modified nucleic acid fragment, which corresponds to a region in the sequence shown in SEQ ID NO:1 preferably has a homology of not less than 70%, more preferably not less than 85%, more preferably not less than 95% with the sequence shown in SEQ ID NO:1. Further, these modified nucleic acid fragments preferably hybridize with the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 1 under stringent condition (i.e., hybridization is carried out in an ordinary hybridization solution such as 5 x Denhardt's reagent, 6 x SSC, 0.5% SDS or 0.1% SDS, at 50 to 65°C, preferably in two steps at 50°C and at 60°C, or in four steps at 50°C, 55°C, 60°C and 65°C).

When inserting the nucleic acid according to the present invention into a site upstream of a structural gene of which expression is desired to be promoted, it is preferred to insert a fragment whose size is as small as possible, which fragment has an activity to promote gene expression. Thus, the number of nucleotides in the

nucleic acid fragment according to the present invention is preferably not more than 120, more preferably not more than 80, and more preferably not more than 64.

By ligating two or more fragments according to the present invention, the activity may be increased. In this case, the nucleic acid fragments according to the present invention may be directly ligated or an intervening sequence may exist therebetween.

The nucleic acid according to the present invention may be either DNA or RNA. However, DNA is preferred in view of stability.

The nucleic acid fragments according to the present invention may easily be prepared by chemical synthesis. Alternatively, since the nucleotide sequence of the first intron of rice PLD gene is known (International Publication WO96/30510), the nucleic acid fragments according to the present invention may easily be obtained by nucleic acid amplification methods such as PCR using the genomic DNA of rice as a template. PCR is well-known in the art and a kit and apparatus therefor are commercially available, so that it can be easily carried out.

In cases where a plurality of nucleic acid fragments according to the present invention are ligated, a plurality of nucleic acid fragments according to the present invention may be preliminarily ligated, or a nucleic acid fragment according to the present invention may be inserted into a region containing the nucleic acid fragment according to the present invention.

By inserting the above-described nucleic acid fragment according to the present invention to a site upstream of a structural gene, the expression of the structural gene may be promoted. Structural genes are controlled by a promoter located upstream thereof. The nucleic acid fragment according to the present invention may be inserted either between the promoter and the structural gene or at a site upstream of the promoter, and the former is preferred. In this case, the distance between the nucleic acid fragment according to the present invention and the

structural gene may preferably be 0 bp to 1000 bp, and the distance between the promoter and the nucleic acid fragment according to the present invention may also preferably be 0 bp to 1000 bp.

It is preferred to insert the nucleic acid fragment according to the present invention into an intron sequence located upstream of the structural gene of which expression is to be promoted. Although such an intron sequence is not restricted, a preferred example is the first intron (SEQ ID NO: 3) of rice PLD gene. In cases where the nucleic acid fragment according to the present invention is inserted into an intron sequence, the site of insertion is not restricted. A part of a primer may be inserted together with an intron fragment. However, in cases where the intron is the first intron (SEQ ID NO: 3) of rice PLD gene, it is preferred to insert the nucleic acid fragments according to the present invention into the site of 1 nt or 65 nt so that a plurality of the nucleic acid fragments according to the present invention are ligated. It is especially preferred to insert the nucleic acid fragment according to the present invention into the site of 65 nt so as to directly ligate two nucleic acid fragments according to the present invention. Although there are cases where an intron sequence does not exist upstream of the structural gene of which expression is to be promoted, in cases where an appropriate intron sequence does not exist, an appropriate intron sequence such as the first intron of rice PLD gene is firstly inserted to a site upstream of the structural gene of which expression is to be promoted, and then the nucleic acid fragment according to the present invention may be inserted therein. Insertion may easily be carried out by a conventional method using one or more restriction enzymes.

The present invention also provides recombinant vectors obtained by applying the above-described method of the present invention to an expression vector. The recombinant vector according to the present invention may easily be prepared by inserting the nucleic acid fragment according to the present invention and a structural

gene of which expression is to be promoted into a cloning site of a commercially available expression vector. Such an expression vector may preferably be one for plants. Various expression vectors for plants are well-known in the art and commercially available. These expression vectors include a replication origin for
 5 replication in host cells, a promoter, cloning sites giving restriction sites for inserting foreign genes, and a selection marker such as a drug resistant gene, and usually contain a terminator which stably terminates transcription. In the method of the present invention, any of these known expression vectors may be employed.

Examples

10 The present invention will now be described more concretely by way of examples thereof. It should be noted that the examples are presented for the illustration purpose only and should not be interpreted in any restrictive way.

Into a vector pBI221 commercially available from CLONTECH, containing beta-Glucuronidase (GUS) gene downstream of 35S promoter (pBI221 (35S
 15 promoter, GUS)), a part of the inner region of the PLD intron, the PLD intron or the PLD intron plus a part of the PLD intron was inserted, and effect of promoting GUS expression was investigated.

The vectors were prepared by the following method. The first intron of rice PLD gene consists of 173 nucleotides (SEQ ID NO: 3). DNA fragments each of
 20 which corresponds to 2nt-65nt, 66nt-120nt or 121nt-173nt of the intron were prepared by PCR. The primers used were as follows:

5'-CTATGACCCGGGATCCTAAGCCCCAGTGTGC-3' and

5'-GCAAGCAAGCAGATCTGAGCGGAGAAGAAG-3';

25 5'-TATGACCCGGGATCCGATCTGCTTGCTTGC-3' and

5'-ACCTAACGTAGATCTAGCGACACTCGCAGC-3';

5'-TATGACCCGGGATCCGCTTCGTCTTCCTTC-3' and

5'-GTGTCGCTAGATCTCTGCGCCCCCCCACAC-3'

Each of the PCR products was digested with restriction enzymes Bam HI and Bgl II, and then inserted into the Bam HI site in the multicloning site in pBI221 to obtain recombinant vectors (pBI[PLD(2-65)], pBI[PLD(66-120)] and pBI[PLD(121-173)]).

Further, vectors further containing the region of 2nt-65nt or 66nt-120nt of the PLD intron in addition to the PLD intron were prepared as follows: First, as described in WO96/30510, the first intron of rice PLD gene (SEQ ID NO: 3) was amplified by PCR using primers (5'-ACCCGGTAAGCCACAG-3', 3'-

CCCCCGCGTCCATCC-5'), and the amplified product was subcloned into pCRII vector. The resultant was digested with Eco RI and the cut out fragment was blunted with Klenow fragment, followed by inserting the blunted fragment into the Sma I site of pBI221 vector to obtain a vector (pBI[PLD]). The intron sequence was cut at its 65nt with Bgl II, and the above-mentioned PCR product digested with Bam HI and Bgl II was inserted thereinto to obtain vectors (pBI[PLD+PLD(2-65)] and pBI[PLD+PLD(66-120)]).

By the reported method (Shimamoto et al. Nature, 338,274-276 (1989)), each of the above-described recombinant vectors was introduced into rice cultured cells (Baba et al. Plant Cell Physiol. 27,463-471 (1986)), and β -glucuronidase (GUS) activity was measured. The relative activities are shown in Table 1.

Table 1

Vector	Relative GUS Activity
pBI221	1.0
pBI[PLD]	14
pBI[PLD(2-65)]	4.9
pBI[PLD(66-120)]	2.5
pBI[PLD(121-173)]	1.7
pBI[PLD+PLD(2-65)]	28
pBI[PLD+PLD(66-120)]	14

All of the three regions which are the parts of the PLD intron exhibited GUS

activities higher than that of the control (pBI221). The region of 2nt-65nt showed the highest activity and the region of 66nt-120nt showed the second highest activity. As for the cases where each of these two regions was inserted into the intron, the activity was twice of the original activity attained by the intron alone in the case of inserting the region of 2nt-65nt into the intron, while the activity was not increased when the region of 66nt-120nt was inserted.

These results revealed that the region of 2nt-65nt of the PLD intron has an activity to promote gene expression. The nucleotide sequence of the region of 2nt-65nt is shown in SEQ ID NO:1, the nucleotide sequence (containing 10 nucleotides each at the both ends) of the intron into which the region of 2nt-65nt is further inserted is shown in SEQ ID NO:4, and the nucleotide sequence in which the exon sequences at the both ends are removed is shown in SEQ ID NO: 2.

10

CLAIMS

1. An isolated nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO: 1 in Sequence Listing or an isolated nucleic acid fragment (excluding the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 3 in Sequence Listing) having the same nucleotide sequence as shown in SEQ ID NO: 1 except that one or a plurality of nucleotides are substituted or deleted, or except that one or a plurality of nucleotides are inserted or added, which has an activity to promote expression of a structural gene located downstream of said nucleic acid fragment.
2. The nucleic acid fragment according to claim 1, which hybridizes with the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 1 in Sequence Listing under stringent condition.
3. The nucleic acid fragment according to claim 1 or 2, which contains nucleotides of not more than 120.
4. The nucleic acid fragment according to claim 1, which has the nucleotide sequence shown in SEQ ID NO: 1 in Sequence Listing.
5. A nucleic acid fragment comprising a plurality of nucleic acid fragments according to any one of claims 1-4, which are ligated.
6. A recombinant vector comprising at least a nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO: 1 in Sequence Listing or a nucleic acid fragment (excluding the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 3 in Sequence Listing) having the same nucleotide sequence as shown in SEQ ID NO: 1 except that one or a plurality of nucleotides are substituted or deleted, or except that one or a plurality of nucleotides are inserted or added, which has an activity to promote expression of a structural gene located downstream of said nucleic acid fragment, and a structural gene located downstream of said nucleic acid fragment, whose expression is promoted by said nucleic acid fragment.
7. The recombinant vector according to claim 6, wherein said nucleic acid

fragment hybridizes with the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 1 in Sequence Listing under stringent condition.

8. The recombinant vector according to claim 6 or 7, wherein said nucleic acid fragment contains nucleotides of not more than 120.

5 9. The recombinant vector according to claim 8, wherein said nucleic acid fragment has the nucleotide sequence shown in SEQ ID NO: 1 in Sequence Listing.

10. The recombinant vector according to any one of claims 6 to 9, wherein said nucleic acid fragment is inserted in an intron sequence located upstream of said structural gene.

10 11. The recombinant vector according to claim 10, wherein said intron sequence has the nucleotide sequence shown in SEQ ID NO: 3 in Sequence Listing.

12. The recombinant vector according to claim 10, wherein said intron sequence has the nucleotide sequence shown in SEQ ID NO: 2 in Sequence Listing.

13. A method for promoting expression of a structural gene, comprising inserting,
15 at a location upstream of said structural gene, a nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO: 1 in Sequence Listing or a nucleic acid fragment (excluding the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 3 in Sequence Listing) having the same nucleotide sequence as shown in SEQ ID NO: 1 except that one or a plurality of nucleotides are substituted or deleted,
20 or except that one or a plurality of nucleotides are inserted or added, which has an activity to promote expression of a structural gene located downstream of said nucleic acid fragment.

14. The method according to claim 13, wherein said nucleic acid fragment hybridizes with the nucleic acid having the nucleotide sequence shown in SEQ ID
25 NO: 1 in Sequence Listing under stringent condition.

15. The method according to claim 13 or 14, wherein said nucleic acid fragment contains nucleotides of not more than 120.

16. The method according to claim 15, wherein said nucleic acid fragment has the nucleotide sequence shown in SEQ ID NO:1 in Sequence Listing.

17. The method according to any one of claims 13 to 16, wherein said nucleic acid fragment is inserted in an intron sequence located upstream of said structural gene.

18. The method according to claim 17, wherein said intron sequence has the nucleotide sequence shown in SEQ ID NO: 3 in Sequence Listing.

19. The method according to any one of claims 13 to 18, wherein a region in which a plurality of said nucleic acid fragments which are ligated is formed by inserting said nucleic acid fragments.

20. A plant or progeny thereof, in which expression of a desired structural gene is promoted by the method according to any one of claims 13 to 19.

ABSTRACT

Novel nucleic acid fragments having activities to prominently promote expression of structural genes located downstream thereof are disclosed. The nucleic acid fragment according to the present invention is an isolated nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO: 1 in Sequence Listing or an isolated nucleic acid fragment (excluding the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 3 in Sequence Listing) having the same nucleotide sequence as shown in SEQ ID NO: 1 except that one or a plurality of nucleotides are substituted or deleted, or except that one or a plurality of nucleotides are inserted or added, which has an activity to promote expression of a structural gene located downstream of said nucleic acid fragment.

1/4

SEQUENCE LISTING

<110> JAPAN TOBACCO INC.

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method for promoting expression of structural genes using the same

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0760-0281P

**COMBINED DECLARATION AND POWER OF ATTORNEY
FOR PATENT AND DESIGN APPLICATIONS**

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: "Nucleic Acid Fragments, Recombinant Vectors Containing the Same and Method for Promoting Expression of Structural Genes Using the Same".

the specification of which is attached hereto. If not attached hereto, _____ as
the specification was filed on _____
United States Application Number _____ (if applicable) and/or
and amended on _____ as PCT
the specification was filed on 09/24/99
International Application Number PCT/JP99/05221
amended under PCT Article 19 on _____; and was
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representative or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)**Priority Claimed**

329832/98	Japan	11/19/98	Yes
(Number)	(Country)	(Month/Day/Year Filed)	
(Number)	(Country)	(Month/Day/Year Filed)	

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More than 12 Months (6 Months for Designs) Prior to the Filing Date of This Application:

Country	Application Number	Date of Filing (Month/Day/Year)
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I hereby claim the benefit under Title 35, United States Code, §120 of any United States and/or PCT application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States and/or PCT application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)
(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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